

## Molecular alterations of *KIT* and *PDGFRA* in GISTs. An evaluation study of a Portuguese series

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## **Abstract**

**Aim:** Assessment of *KIT* and *PDGFRA* mutations frequencies in a Portuguese series of gastrointestinal stromal tumours (GISTs).

**Methods and Results:** Seventy-eight GISTs were evaluated for CD117 expression and screened for mutations in *KIT* (exons 9, 11, 13, 14 and 17), and *PDGFRA* (exons 12, 14 and 18) genes. *KIT* activating mutations were identified in 44 (56%) of the 78 GISTs. Forty cases (91%) presented a mutation in *KIT* exon 11 and 4 (9%) in exon 9. One case showed a 4 bp deletion in intron 14. *PDGFRA* mutations were observed in cases 5 (6%), 2 (3%) in exon 12 and 3 (4%) in exon 18.

Survival analysis was performed in 63 of the 78 GISTs. The presence of mutated *KIT* was significantly correlated with shorter survival of patients ( $P=0.0460$ ), and inversely associated with epithelioid histological type of GISTs ( $P=0.0064$ ).

**Conclusions:** Overall, the incidence of both *KIT* and *PDGFRA* mutations in these Portuguese series was 63%, being in agreement with other studies, mainly of Iberian populations. The great majority of mutations were located in *KIT* exon 11, statistically associated with worse prognosis and indicative of favourable response to Imatinib-based therapy in this Portuguese series of GISTs.

## **Take home messages**

- We described for the first time the frequency of *KIT* and *PDGFRA* activating mutations in a series of Portuguese GISTs.
- Fifty-six percent of Portuguese patients with GISTs harboured *KIT* mutations and six percent exhibited *PDGFRA* mutations.
- The presence of *KIT* mutations in GISTs was associated with a worse patient prognosis; however, these mutations are indicative of favourable response to Imatinib-based therapy.

## Introduction

Gastrointestinal stromal tumours (GISTs) although rare, are considered to be the most frequent gastrointestinal mesenchymal tumours in humans.<sup>1</sup> A Scandinavian study, estimated the incidence of GISTs to be between 20 and 40 per million.<sup>2</sup> In Portugal, as far as we know, an epidemiologic study is yet to be done.

GISTs cellular origin is not fully understood, but they are thought to arise from interstitial cells of Cajal or their precursors, due to their similar positive KIT (CD117) and CD34 staining and negative staining for both desmin and S-100 protein immunostaining.<sup>1 3</sup> GISTs are rarely found outside the gastrointestinal tract, being most commonly found in the stomach (40 to 70%), small intestine (20 to 50%), and colon or rectum (5 to 15%).<sup>1 2 4</sup> Nowadays, the diagnosis of GISTs is partially dependent on tumour cells overexpression of CD117 together with CD34.<sup>5</sup> The expression of such immunohistochemistry features is much useful to differentiate GISTs from other mesenchymal tumours of the gastrointestinal tract, namely leiomyomas and leiomyosarcomas, nerve sheath tumours, and other primary and metastatic tumours possibly occurring in this location.<sup>1 2 4</sup>

KIT belongs to the class III receptor tyrosine kinases (RTKs), which also includes the platelet-derived growth factor A and B (PDGFRA, PDGFRB), the colony stimulating factor-1 receptor (CSF1R) and the FMS-related tyrosine kinase 3 (FLT3).<sup>6</sup> These RTKs are characterized by the presence of an extracellular domain, a transmembrane domain, a juxtamembrane domain, and an intracellular domain where the two kinase domains are lodged.<sup>7</sup> RTK activation occurs when by ligand binding, the receptor dimerizes and suffers conformational transformations, which induce the activation of the kinase domains. These, in turn, lead to the activation of important intracellular signalling pathways, such as RAS/mitogen activated protein kinase (RAS/MAPK), phosphoinositide-3 kinase (PI3K), and signal transducers and activators of transcription (STAT), which regulate many physiological functions such as cell survival, proliferation, differentiation, adhesion and apoptosis.<sup>7-8</sup>

GISTs are molecularly characterized by mutations in *KIT* oncogene, located in the long arm of chromosome 4 (4q11-q12).<sup>9</sup> There is a broad spectrum of *KIT* mutations in GISTs, ranging from 20 up to 80 %, being most of them located in the juxtamembrane domain (exon 11), followed by mutations in the extracellular domain (exon 9), and seldom in the kinase (exon 13 and 17) and ATP pocket (exon 14) domains.<sup>10-12</sup> Later studies reported the presence of activating mutations in *PDGFRA* oncogene in wild-type *KIT* bearing GISTs.<sup>13-14</sup> *PDGFRA* is also located at 4q11-12 and exhibits similar RTK cellular functions.<sup>13-14</sup> The hotspot regions in this gene lay in the juxtamembrane (exon 12) and in the kinase (exons 14 and 18) domains, and have been reported in 5-12% of cases.<sup>12 14</sup> The frequency of *KIT/PDGFRA* mutations in GISTs varies from series to series, probably reflecting epidemiological and methodological differences in the various studies on record.<sup>10 12</sup>

Until recently, the treatment of GISTs was limited to surgical removal of the tumour. Unfortunately, even in patients where the tumour was completely and successfully removed, there was a high probability of recurrence.<sup>1</sup> The development of Imatinib mesylate (Glivec®/Gleevec™, Novartis), a selective inhibitor of RTKs, has brought new hope for GISTs patients. Imatinib targets KIT by competing with its ATP binding site, preventing further phosphorylations of

downstream intracellular signalling molecules responsible for its oncogenic properties.<sup>15-16</sup> Several studies have showed the importance of *KIT* and *PDGFRA* molecular status in Imatinib response.<sup>10 12</sup> It has been reported that patients with tumours harbouring exon 11 *KIT* mutations are more likely to respond to an Imatinib therapy than those with either exon 9 *KIT* mutations or undetectable mutations.<sup>10 12</sup>

In Portugal, the incidence of both *KIT* and *PDGFRA* mutations in GISTs tumours is, to the best of our knowledge, unknown. Since different genotypic features give rise to different drug responses and thus different prognosis, it becomes important to define which patients will positively respond to Imatinib treatment. Therefore, we characterized the occurrence of *KIT* and *PDGFRA* mutations in a series of Portuguese GISTs patients.

## Materials and methods

### Tissue samples

Seventy-eight formalin-fixed and paraffin-embedded *bona fide* consecutively diagnosed primary previously untreated sporadic gastrointestinal stromal tumours (GISTs), classified according to WHO<sup>5</sup> and risk group,<sup>17</sup> were retrieved from the Pathology Department of S. João Hospital files (1989-2005), Porto, Portugal. All patients were Caucasian of Portuguese origin, with a mean age of 61.7 years (range 20-88yr). Thirty-eight (48.7%) patients were females and 40 (51.3%) were males. Follow-up data, managed according to the guidelines of the European Society of Medical Oncology,<sup>18</sup> was available in 63 patients (range: 0.2-206.0 months, mean: 122.8 ± 12.1 months, median: 132.6 ± 26.8 months), as at September 2006.

### Immunohistochemistry

Immunohistochemistry procedure was performed according to streptavidin-biotin-peroxidase complex principle, using rabbit polyclonal anti-human antibodies raised against CD117 (dilution 1:500; clone A 4502, DAKO A/S, Denmark), actin (dilution 1:100; clone HHF35, DAKO, A/S, Denmark), desmin (dilution 1:50; Zymed Laboratories, S. San Francisco, CA), S100 protein (dilution 1:1000; DAKO A/S, Denmark); endothelial cell marker CD34 (dilution 1:40; clone QBEnd/10, NovoCastra Laboratories Ltd). Briefly, deparaffinized and rehydrated slides were subjected to 10 minutes incubation in 3% hydrogen peroxide in methanol, in order to inhibit endogenous peroxidase. No antigen retrieval was used. After incubation with primary antibody at room temperature for 30 minutes, the secondary biotinylated goat anti-polyvalent antibody was applied for 10 minutes, followed by incubation with streptavidin-peroxidase complex. The immune reaction was visualized by DAB as a chromogen (Ultravision Detection System Anti-polyvalent, HRP/DAB; Lab Vision, Fremont, CA). Any (strong/weak, focal, moderate or diffuse) membrane (CD117) and/or cytoplasm (CD117, actin, desmin, and CD34), and nuclear (S100 protein) immunoreactivity of the cells was considered as positive staining. Appropriated positive and negative controls were included in each run: interstitial cells of Cajal in a section of normal intestine were used as positive control for CD117, smooth layers for actin and desmin, small nerves for S100 protein, and vessels for CD34. For negative controls, primary antibodies were omitted. Mast cells, smooth layers, small nerves, and vessels were used as internal positive controls in the cases tested. All sections were counterstained with haematoxylin.

### DNA isolation

Selected areas containing at least 85% of tumour tissue were macrodissected into a microfuge tube using a sterile needle (Neolus, 25G- 0.5mm). DNA isolation was performed as previously described.<sup>19</sup> Briefly, the dissected tissue was deparaffinized by a serial extraction with xylol and ethanol (100%-70%-50%) and allowed to air-dry. DNA was extracted using Qiagen's QIAamp® DNA Micro Kit, following the manufacturer's instructions. DNA samples were stored at -20°C for further analysis.

### *KIT* mutation analysis

*KIT* mutation analysis was performed as previously described.<sup>19-20</sup> DNA was subjected to PCR amplifications followed by direct sequencing for exon 11, and pre-screening by single strand conformational polymorphism (SSCP) analysis for exons 9, 13, 14 and 17, followed by direct sequencing of SSCP positive cases. Briefly, the PCR reaction was carried in a final volume of 25µL, under the following conditions: 1x Buffer (Bioron, Germany); 1.5mM MgCl<sub>2</sub> (Bioron, Germany); 200µM dNTPs (Fermentas, USA); 0.5µM primers [previously described by Corless *et al*,<sup>21</sup> except for exon 14: 5'-TCTCACCTTCTTTCTAACCTTTTCTT-3' (forward); 5'-CCCATGAACTGCCTGTCAAC-3' (reverse); MWG-Biotech, Ebersberg, Germany] and 1 unit of Super Hot Taq Polymerase (Bioron, Germany). SSCP analysis of exons 9, 13, 14 and 17 was performed in a 1X MDE gel (MDE: mutation detection enhancement, Cambrex, USA), with 6% glycerol addition in the exon 13 analysis, and 3% glycerol addition in exon 14 analysis. Twenty microlitres of PCR product were incubated at 95°C for 10 minutes with an equal volume of formamide loading buffer (98% formamide, 10mM EDTA, and 1mg/mL bromophenol blue and xylene cyanol). SSCP gels were run at 20°C. Samples with a SSCP pattern different from the normal pattern were directly sequenced. All cases were confirmed twice with a new PCR amplification, SSCP and direct sequencing analysis.

#### ***PDGFRA* mutation analysis**

Tumours bearing a wild type *KIT* gene were further screened for hotspot *PDGFRA* mutations (exons 12, 14 and 18) as previously described.<sup>19-20</sup> Briefly, the PCR reaction was carried out in a final volume of 25µL, under the following conditions: 1x Buffer (Bioron, Germany); 1.5mM MgCl<sub>2</sub> (Bioron, Germany); 200µM dNTPs (Fermentas, USA); 0.5µM primers (previously described by Heinrich *et al*,<sup>13</sup> MWG-Biotech, Ebersberg, Germany) and 1 unit of Super Hot Taq Polymerase (Bioron, Germany). PCR was followed by direct sequencing. All cases were confirmed twice with a new PCR amplification and direct sequencing analysis.

#### **Statistical analysis**

The available clinical and molecular data were analysed with StatView software for Windows, version 5.0. Overall survival time analysis using Kaplan-Meier and Log rank tests were done with SPSS software for Windows, version 14.0. *P* value inferior to 0.05 was considered significant.

## Results

### Immunohistochemistry

Strong membrane and/or cytoplasm tumour cells immunoreactivity for CD117 was found in variably focal, moderate or diffuse areas in 72 (92%) of GIST cases (Figure 1). In three cases, CD117 immunoreactivity was weak. Six GIST cases (8%) did not disclose CD117 immunoreactive tumour cells. Interstitial cells of Cajal and mast cells, used as internal positive controls, were always variably observed in each case.

Statistical analysis of CD117 immunostaining and clinical-pathological features are summarized in Table 1. CD117 immunoreactivity was significantly associated ( $P = 0.0151$ ) with spindle cell and epithelioid GIST subtypes.

The frequency and expression features of the other antibodies was variable from case to case, and within the same tumour, as follows: actin (51%), desmin (6%), S100 protein (18%), and CD34 (73%); the immunoreactivity was observed in focal areas/rare tumour cells for actin, desmin, and S100 protein, whereas CD34 immunoreactivity was found in moderate or diffuse areas (data not shown). Four of the six CD117 negative GISTs expressed CD34 without any tumour cell expression for the other markers tested.

### *KIT* Mutation Analysis

Mutation screening analysis revealed that 44 out of 78 GISTs (56%) presented *KIT* activating mutations (Table 2). Forty cases showed mutation in exon 11 (91%, 40/44) and four cases in exon 9 (9%, 4/44). Among the exon 11 mutations, we observed 3 to 54 bp in-frame deletions in 24 tumours (60%, 24/40), either alone (62%, 15/24) or associated with missense mutations or insertions (38%, 9/24), single base substitutions in 15 tumours (38%, 15/40) and an in-frame insertion associated with a point mutation in 1 tumour (2%, 1/40). Additionally, a silent mutation (Y570Y) was detected in two GISTs. The exon 9 sequence alterations consisted of Ala-Tyr duplication between codons 502-503 in three GIST cases, and a point mutation (G470R) in one case. One silent mutation was detected in both exons 13 and 17 (P627P, and S865S, respectively). Also, a 4bp deletion was detected affecting the intronic sequence following exon 14 (IVS14+24:del4). In addition, to exclude the possibility of false-negatives in the SSCP screening at exons 9, 13, 14 and 17, ten *KIT* wild type GISTs were direct sequenced for all exons. No additional mutations were identified.

Statistical analysis of *KIT* mutations and clinical-pathological features are shown in Table 3. No correlation was detected between *KIT* mutation status and CD117 expression ( $P=0.3933$ ). However, all but two GISTs harbouring *KIT* mutation were positive for CD117 expression. Additionally, the 3 GISTs with weak CD117 immunoreactivity depicted wild-type *KIT*. A statistically significant correlation was obtained between the epithelioid morphology and lack of *KIT* mutation ( $P=0.0064$ ). The presence of mutated *KIT* was significantly associated with shorter survival of patients ( $P=0.0460$ ) (Figure 2). No correlation was obtained between any specific type of *KIT* mutation (point mutation, deletion, or mixed mutation), or its location (exon 9, or exon 11), and patient survival (data not shown).

### *PDGFRA* Mutation Analysis

In *KIT* wild-type GISTs, *PDGFRA* activating mutations were identified in 5 cases; two in exon 12, and three in exon 18 (Table 4). The mutations in exon 12 consisted of a point mutation (D583G) and an in-frame deletion (583del586); two cases disclosed a point mutation (D842V) in exon 18, and another case with a point mutation (I843T) together with an in-frame deletion (844del847). In addition, we identified two silent mutations, one in exon 12 (D577D) and another in exon 18 (I834I). No mutation was observed in exon 14 of *PDGFRA*. Furthermore, mutational analysis of exons 12 and 18 showed the presence of a known homozygous substitution A>G (polymorphism R) in the third position of the codon for Proline 567 in exon 12, and an insertion in the intron 18 (IVS18-50insA).

All GISTs with *PDGFRA* mutations showed CD117 immunoreactive tumour cells.

## Discussion

The intensive cancer research in the last decade highlighted the fundamental role of RTKs, in particular of KIT and PDGFRA in GISTs pathogenesis.<sup>10</sup> These two RTKs are nowadays of great value for therapeutic management given the development of RTK inhibitors, such as Imatinib and Sunitinib.<sup>22-24</sup> There are not enough epidemiological data yet on the frequency and type of mutations in the *KIT* and *PDGFRA* genes in GISTs from South Europe countries, namely Portugal.

In this study, we have shown that 92% of GISTs express CD117, irrespective of the topography, age or gender, in accordance with previous studies in other populations.<sup>25-26</sup> No statistically significant correlation was depicted between CD117 expression and presence of *KIT* mutations ( $P=0.3933$ ). In fact, two of the six CD117-negative GISTs contained a *KIT* mutation (a missense mutation in exon 9, and a 3 base-pair deletion in exon 11). Other authors have also encountered *KIT* mutations in CD117-negative GIST cases.<sup>27</sup> Noteworthy, our molecular study was useful for the definitive diagnosis of GIST in 2 out of 6 CD117 negative cases. The frequency of the CD117-negative cases wild-type for *KIT* and *PDGFRA* mutations found in our series (5%), fits with results described in the literature.<sup>27</sup>

We showed the presence of *KIT* mutations in 56% of GIST cases, being 91% (40/44) located in exon 11. These frequencies are in accordance with previously published ranges for other populations (30-80%), particularly those of Iberian Peninsula.<sup>1 10 28</sup> In 75% (30/40) of these cases, mutations were clustered in the region between codons 550 and 561, known to be the most frequently altered section of exon 11, with 57% (17/30) affecting either codons 557 or 558. These two codons are reported to be associated with GISTs' metastatic behaviour.<sup>10 29</sup> However, of these 17 GIST cases, only four recurred (4/17, 24%). Even though it has been previously described that all point mutations occur exclusively in codons 557, 559, 560, and 576, we have additionally encountered a novel point mutation in codon 570 (Y570F).<sup>1</sup> Mutations in *KIT* exon 9 have been correlated with a small intestinal topography, but only 1 out of our 4 GIST cases harbouring a mutation in this exon was located in the small intestine.<sup>1 10</sup> In the present study, and in agreement with previous reports, *KIT* mutation positive status was shown to be associated with worse GISTs prognosis, translated in shorter patient survival.<sup>10</sup>

Concerning *PDGFRA*, mutations were detected in 6% (5/78) of our cases, corresponding to 15% of *KIT* wild type GISTs. Two of these mutations are known to be Imatinib-resistant mutation (D842V).<sup>22</sup> It has been reported an association between gastric location and presence of *PDGFRA* mutation.<sup>30</sup> In our series, although the number of cases with mutations in *PDGFRA* is low for statistical evaluation (n=5), 80% (4/5) of mutations occurred in the stomach.

It is now well established that GIST patients' response to Imatinib-based therapy is dependent not only on the presence, but also on the type of *KIT* and *PDGFRA* mutation exhibited.<sup>10 16 22</sup> Specifically, mutations affecting the juxtamembrane domain (exon 11, partial response in up to 84% of the cases) or the extracellular domain (exon 9, partial response in up to 48%) predict objective response to Imatinib.<sup>10 16 22</sup> On the other hand, it is also known that some mutations are responsible for Imatinib resistance, namely in *KIT* V654A and W670I (exon 13), D816V and T823D (exon 17), and in *PDGFRA* D842V (exon 18).<sup>16 31-32</sup> Of these resistant mutations, only D842V mutation was

detected in two GISTs of our series. Recently, the U.S. Food and Drug Administration (FDA) approved a new RTK inhibitor, Sunitinib (Sutent®, Pfizer) as a second-line therapy for GIST patients who experience disease progression in spite of increased doses of Imatinib, mainly due to primary or acquired secondary Imatinib-resistant mutations, or who are unable to tolerate treatment with Imatinib.<sup>24 33</sup> Therefore, with these two RTK inhibitors available, there is an imperative need for redefining GIST pathological (diagnosis/prognostic) evaluation, as well as to consider molecular characterization of both *KIT* and *PDGFRA*, in order to achieve an efficient and predictive tailored therapeutic management for each individual patient care.

In conclusion, we reported for the first time the frequency of *KIT* and *PDGFRA* mutations in a large series of Portuguese GISTs patients. We showed the presence of *KIT* mutations in 56% of the cases and *PDGFRA* mutations in 6% of the cases. In addition, the presence of mutated *KIT* was associated with a shorter patient survival. The great majority of *KIT* activating mutations (91%) were located in exon 11, indicative of a favourable response to Imatinib-based therapy in the management of these patients. Finally, our results might be useful to integrate a multi-institutional consortium database for the clarification of the epidemiology, biology and management of GIST patients.

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### Figure legends

Figure 1: Morphological and immunohistochemical features of GISTs: (A) spindle cell and (B) epithelioid tumour cells with (C) membranar/cytoplasmic and (D) cytoplasmic/paranuclear dot immunoreactivity for CD117. Note immunoreactivity of interstitial cells of Cajal (C-inset). H&E and ABC immunohistochemistry (200X).

Figure 2: Kaplan-Meier curve for the 63 GIST patients, regarding *KIT* alterations. Patients having a wild-type *KIT* (n=27, light blue) have a better prognosis than patients having tumours harbouring mutated *KIT* (n=36, dark blue) ( $P=0.046$ ).

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Table 1: Correlation analysis of CD117 with clinical-pathological features of GISTs (n=63).

<b>Parameter (n=63)</b>	<b>CD117 negative (%)</b>	<b>CD117 positive (%)</b>	<b>P value</b>
<b>Age (yrs ±SD)</b>	54.6 ± 12.1	61.2 ± 15.5	0.3735
<b>Gender</b>			
Male	(13.7)	(86.3)	0.1293
Female	(3.3)	(96.7)	
<b>Location</b>			
Gastric	(5.9)	(94.1)	0.1761
Small intestine	(5.0)	(95.0)	
Other	(25.0)	(75.0)	
<b>Dimension (cm)</b>			
<5	(4.0)	(96.0)	0.7639
>5	(5.7)	(94.3)	
<b>Mitotic Index (HPF)</b>			
<5	(11.4)	(88.6)	0.3402
5-10	(0.0)	(100.0)	
>10	(0.0)	(100.0)	
<b>Risk grade</b>			
VLR-LR	(5.0)	(95.0)	0.9324
IR	(6.7)	(93.3)	
HR	(4.0)	(96.0)	
<b>Histological Type</b>			
Spindle cell	(2.5)	(97.5)	0.0167
Epithelioid	(0.0)	(100.0)	
Mixed	(25.0)	(75.0)	
<b>Follow-up (months ±SD)</b>	92.3±32.8	120.8±12.6	0.9630

SD: standard deviation; HFP: high power field (x400); VLR: very low risk; LR: low risk; IR: intermediate risk; HR: high risk

Table 2: Aminoacid sequence of exons 9 and 11 of wild type and mutated KIT protein\*

exon 11	550	560	570	580
Wild type	K P M Y E V Q W K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Cases 5, 67, 76, 78	K P M Y E V Q W K <b>D</b>	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 7	K P M Y E V Q W _ V	V E E I N G N N Y V	Y I D P T Q N P Y D	H K W E F P R N R L
Case 10	K P M Y E V Q W K V	V E E I _ _ _ _ _ V	Y I D P T Q L P Y D	H K W E F P R N R L
Cases 11, 59	K P M Y E V Q W K V	_ E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 13	K _ _ _ E V Q W K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Cases 14, 40	K P M Y E V Q W K V	<b>E</b> E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 15	K P M Y E V Q <b>F</b> _ V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 16	K P M Y E V Q W K V	V E E I N G N N Y V	<b>F</b> I D P T Q L P Y D	H K W E F P R N R L
Case 17	K P M Y E V Q <b>G</b> K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R P
Case 19	K P _ _ _ _ _	_ _ _ _ _	_ I D P T Q L P Y D	H K W E F P R N R L
Cases 22, 39, 57, 70	K P M Y E V Q W K V	V E E I N G N N Y V	Y I D P T Q <b>P</b> P Y D	H K W E F P R N R L
Case 24	K P M Y E V Q W K <b>G</b>	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 28	K P M Y E V Q W K V	V E E I N G N N Y V	Y I D P T <b>H</b> _ <b>T</b> Y D	H K W E F P R N R L
Case 33	K P M Y E V Q W K V	<b>D</b> E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 37	K P M Y E V Q W K _	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Cases 38, 42, 47	K P M Y E V Q _ _ V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 45	<b>Q R</b> _ _ _ _ _ K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 48#	_ _ _ _ _ K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 50	K P M Y E V Q W K V	V E E I _ _ _ _ _	_ _ _ _ _ Y D	H K W E F P R N R L
Case 51	K P M Y E V _ _ K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 55	K P M Y E V Q <b>R</b> K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 64	K P M Y E V Q W <b>N</b> _	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 65	<b>I</b> _ _ _ _ V Q W K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 66	K P M Y E V <b>H</b> _ _ _	_ _ _ _ _	_ _ _ P T Q L P Y D	H K W E F P R N R L
Case 68	K P M Y E V _ _ K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
Case 69	K P M Y E V Q W K V	V E E I N G N <b>K</b> _ _	_ _ _ _ _ <b>V</b> P Y D	H K W E F P R N R L

<b>Case 73</b>	K P M Y E V P _ _ _	_ _ _ E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
<b>Case 74</b>	K P M Y E V Q W K V	V E E I N G N N Y V	Y I D P T Q L P Y D	H P T Q L P Y D H L
<b>Case 75</b>	K P M Y E V Q W _ _	_ _ E E I N G N N Y V	Y I D P T Q L P Y D	H K W E F P R N R L
<b>Case 77</b>	K P M Y E V Q W _ _	_ _ _ _ _ _ _ _ _ _	_ _ _ _ _ P Y D	H K W E F P R N R L

<b>Exon 9</b>	470	...	500							510						
<b>Wild type</b>	F	G	K	...	T	S	A	Y	F	N	F	A	F	K	G	N
<b>Cases 2</b>	F	R	K	...	T	S	A	Y	F	N	F	A	F	K	G	N
<b>Cases 1, 12, 18</b>	F	G	K	...	T	S	A	Y	A	Y	F	N	F	A	F	K

\*, underscore indicates deleted amino acid residues; # included an insertion of two residues (Histidine and Asparagine) in codon 550, besides the represented deletion.

Table 3: Correlation of *KIT* mutations with clinical-pathological features of GISTs (n=63).

<b>Parameter</b>	<b><i>KIT</i> mutation negative (%)</b>	<b><i>KIT</i> mutation positive (%)</b>	<b><i>P</i> value</b>
<b>Age (yrs ±SD)</b>	57.2 ±16.6	62.8±14.2	0.1600
<b>Gender</b>			
Male	(43.3)	(56.7)	0.9419
Female	(42.4)	(57.6)	
<b>Location</b>			
Gastric	(51.4)	(48.6)	0.2731
Small intestine	(35.0)	(65.0)	
Other	(25.0)	(75.0)	
<b>Dimension (cm)</b>			
<5	(44.0)	(56.0)	0.9298
>5	(42.8)	(57.2)	
<b>Mitotic Index (50 HPF)</b>			
<5	(45.4)	(54.5)	0.7985
5-10	(44.4)	(55.6)	
>10	(33.3)	(66.7)	
<b>Risk grade</b>			
VLR-LR	(40.0)	(60.0)	0.7090
IR	(53.3)	(46.7)	
HR	(42.3)	(57.7)	
<b>Histological subtype</b>			
Spindle	(41.5)	(58.5)	<b>0.0064</b>
Epithelioid	(100.0)	(0.0)	
Mixed	(25.0)	(75.0)	
<b>CD117 expression</b>			
positive	(40.4)	(59.6)	0.3933
negative	(60.0)	(40.0)	
<b>Follow-up (months ±SD)</b>	148.7±17.1	100.8±12.1	<b>0.0460</b>

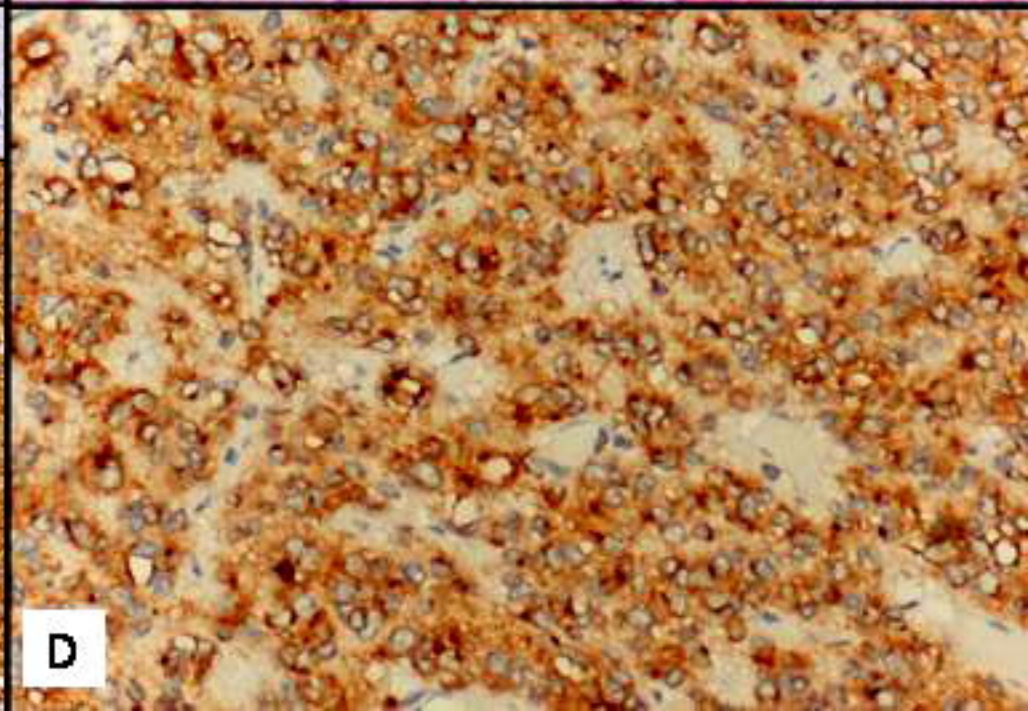
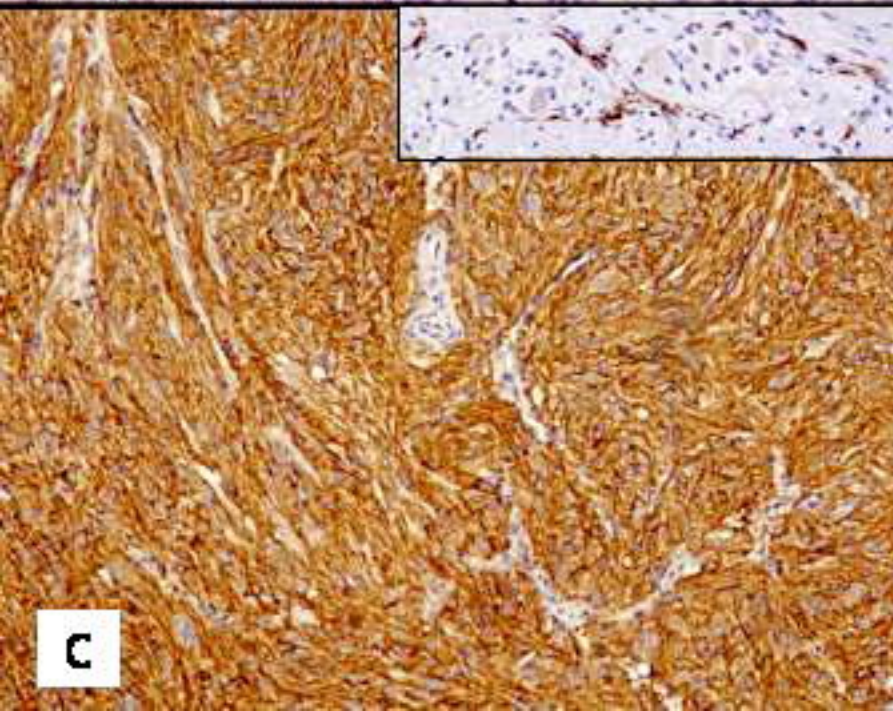
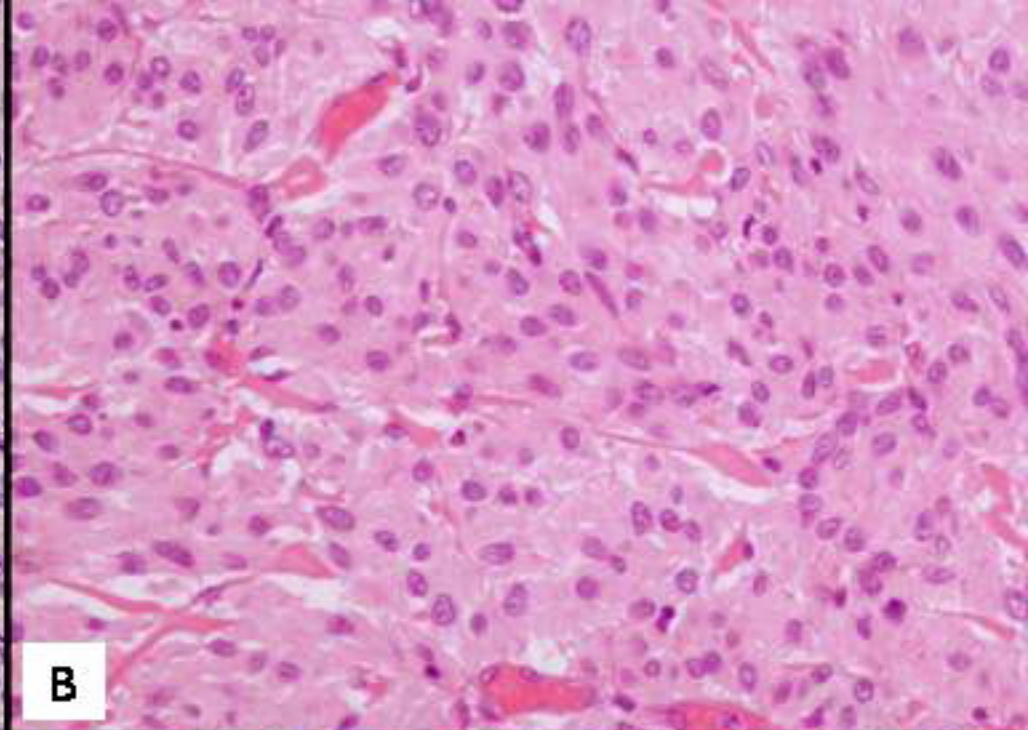
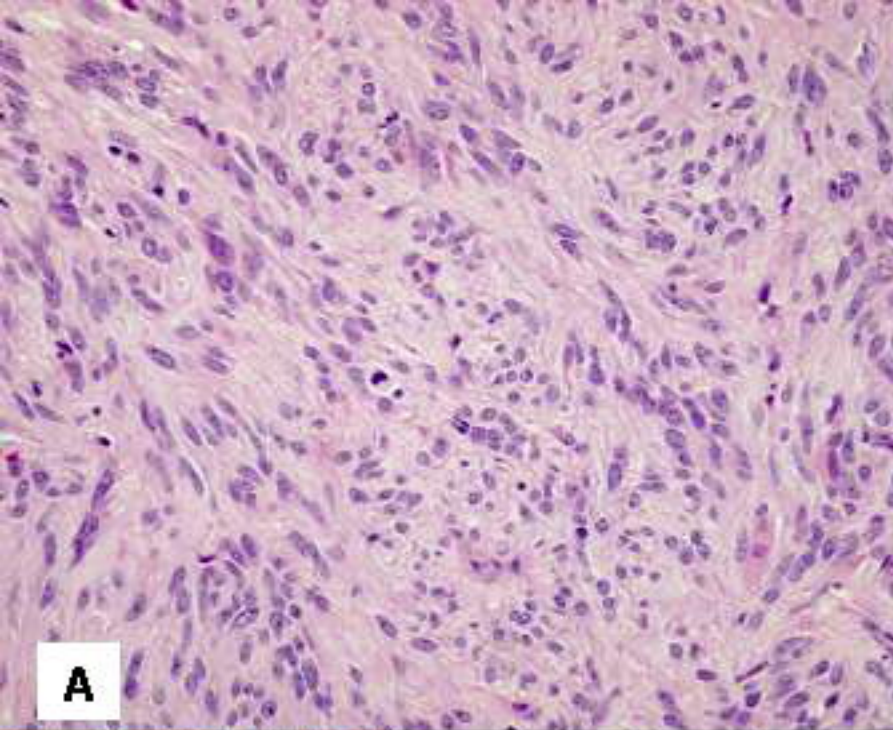
SD: standard deviation; HFP: high power field (x 400); VLR: very low risk; LR: low risk; IR: intermediate risk; HR: high risk

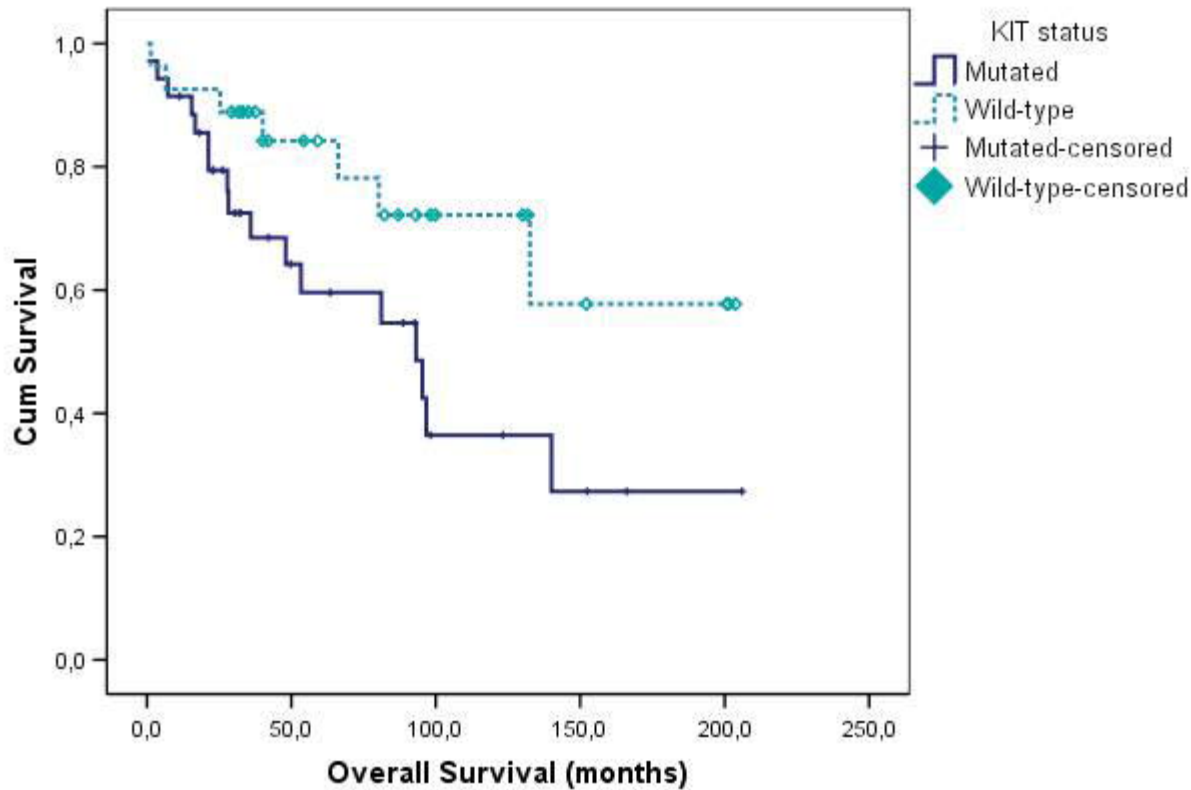
Table 4: Aminoacid sequence of exons 12 and 18 of wild type and mutated PDGFRA protein\*

<i>Exon 12</i>	<b>580</b>									
<b>Wild type</b>	L	P	Y	D	S	R	W	E	F	P
<b>Case 4</b>	L	P	Y	G	S	R	W	E	F	P
<b>Case 71</b>	L	P	Y	-	-	-	-	E	F	P

<i>Exon 18</i>	<b>840</b>									
<b>Wild type</b>	A	R	D	I	M	H	D	S	N	Y
<b>Cases 41,54</b>	A	R	V	I	M	H	D	S	N	Y
<b>Case 34</b>	A	R	D	T	-	-	-	-	N	Y

\*Underscore indicates deleted amino acid residues







## Molecular alterations of KIT and PDGFRA in GISTs. An evaluation study of a Portuguese series

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